JJC

Volume 13, Number 2, June 2018
Pages 69-91

Jordan Journal of Chemistry

ARTICLE

Assessment of Film-Forming Agents in Water-Based Paints

Ajoke F. Apanpa-Qasim[a,b*], **Adebola A. Adeyi**[a] and **Rashmi Dixit**[b]

[a] *Department of Chemistry, University of Ibadan, Ibadan-Nigeria.*
[b] *CSIR-National Environmental Engineering and Research Institute, Nagpur, India.*

Received on: 7th Nov. 2017; Accepted on: 21st Jan. 2018

Abstract: Film-forming agents (FFAs) are volatile organic compounds used in paints and are of global health concern. In this study, 174 paint samples manufactured by 14 different paint manufacturers and marketed in Lagos and Ibadan, Nigeria were purchased. Samples were solvent-extracted with acetonitrile and the FFAs present were identified and subsequently quantified using GC-MS and GC-FID, respectively. Biodegradation study of the most commonly used FFAs was also carried out. Ethylene glycol butyl ether (EGBE), diethylene glycol butyl ether (DEGBE), ethylene glycol butyl ether acetate (EGBEA) and ethylene glycol ethyl ether acetate (EGEEA) were present in the paint samples. The FFA levels in all studied paint samples ranged from 1,010-3,350 ppm of DEGBE, 1,000-1,860 ppm of EGBE, 1,120-3,870 ppm of EGBEA and 1,020-2,900 ppm of EGEEA. The levels of these FFAs were above the European Union (EU) permissible limits of 500 ppm for volatile organic compounds (VOCs) in paints. Therefore, water-based paints marketed in Nigeria contain high concentrations of these FFAs, which are of human health concern and hazardous to the environment. During the biodegradation of EGBEA, the stationary phase was observed at 20 ppm (cell growth rate equals cell death rate). This implies a possible bioaccumulation over time. Also, biodegradation of EGBEA fitted into a typical Monod kinetics model for substrate utilization.
Keywords: Water-based paints, Film-forming agents, Biodegradation, Gas chromatography, Human exposure.

Introduction

Various symptoms, such as dry mucous membranes and dry skin, irritation of eyes, nose and throat, chest tightness, headache and mental fatigue, have been reported by people living in modern buildings[1]. These nonspecific health problems, mostly associated with indoor environments, are caused by volatile organic compounds (VOCs) emitted from various sources, such as building materials[2-4]. household materials[5,6] and combusted materials[7,8]. Water-based paint (WBP) is a liquid mixture, usually consisting of a solid pigment suspended in a liquid, that when applied to a surface dries to form a hard thin decorative or protective coating. It is a building material composed of pigments, solvents, resins and various additives, some of which are VOCs. The pigments give the paint its colour, solvents make it easier to apply, resins help it dry and additives serve as dryness enhancer and do also provide opacity[9]. Today, paints are used for interior and exterior house painting, boats, automobiles, planes, appliances, furniture and many other materials where protection and appeal are desired[10-12].

Volatile Organic Compounds are organic chemicals that have a high vapour pressure at ordinary room temperature[11,13]. There are several definitions of VOCs, because volatility depends on many different parameters, such as boiling point, vapor pressure, molecular weight and size. EU directive 2004/42/EC defines VOCs as any organic compounds having initial boiling points less than or equal to 250 ºC measured at astandard pressure of 101.3 kPa[14].

This causes large numbers of molecules to evaporate or sublime from the liquid or solid form of the compound and enter the surrounding

• **Corresponding Author:** Ajoke F.I. Apanpa-Qasim **Email**: ajoketola@gmail.com

air. In the course of their use, individuals are frequently exposed to hazardous chemicals, such as volatile organic compounds in both outdoor and indoor environments[15]. However, the major route of exposure is inhalation by workers (occupational exposures) during production and distribution of paint products as well as by the general public during usage.

Some VOCs are dangerous to human health and can cause harm to the environment. Anthropogenic VOCs are regulated by law, especially in indoors, where concentrations are highest. Harmful VOCs are typically not acutely toxic, but instead have compounding long-term health effects, such as immune system disorders, development of cancer, irritation, allergic reactions and so on[16,17]. FFAs (such as texanol and cellosolve)[15] are used in water-based formulations to support the film forming process of paint or coating. They serve as coalescing agents which enable polymer dispersion particles to form a homogeneous film even at low temperatures. The harmful effects of FFAs to humans and to the environment cannot be over-emphasized, as their symptoms are slow to develop and could eventually lead to death. Ethylene glycol monoethyl ether acetate is toxic to the bone marrow[18]; ethylene glycol butyl ether and acetate irritate the eyes and skin and affect the central nervous system, kidneys and liver[19]. There are many studies on VOC measurements in air[20-25]. but very few directly analyzed VOCs in water based-paints[15,26-28].

Gas chromatography has become a highly recommended tool for monitoring and tracking organic pollutants, such as VOCs in consumer products and the environment[29-32]. It is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes, … etc[33,34]. It is also used in forensic and criminal cases[35,36] as well as in industrial and academic research.

There is a need to identify and quantify VOCs (FFAs) in consumer products, such as paints sold in Nigeria, because of the effects they pose on human health and the environment.

There are no regulations in place in the country to control these VOCs in paints. This study identifies and assesses the levels of FFAs in paint samples marketed in Nigeria and carries out a biodegradation study on the toxic and most commonly used FFAs in the product to ascertain their fate in the environment. The study also compares the levels in the samples with the available permissible limit as regulated by the European Union.

Materials and Methods

Sample Collection and Preparation

Most commonly used water-based paint samples were purchased from different paint markets in Ibadan and Lagos, Nigeria based on colour availability (Table 1). Six registered manufacturers with NIS-Nigerian Industrial Standard, ISO–International Organization for Standardization certification and 8 unregistered manufacturers without NIS-ISO certification were selected. A total of 174 paint samples produced by 14 different manufacturers were collected, stored in air-tight plastic containers and analyzed at the Council of Scientific and Industrial Research- National Environmental Engineering Research Institute Laboratory, Nagpur – Maharashtra, India.

About 2.5 mL of paint samples were carefully measured into 50 mL polypropylene radiation sterilized centrifuge tubes and extracted using liquid-liquid extraction with milli-Q water followed by acetonitrile in the ratio 3:4 (v/v). The centrifuge tubes were shaken and mixed on a cyclo-mixer at 50 cycles (CM 101) for sample homogeneity. The tubes were centrifuged at 5000 rpm at 20°C for 20 minutes. After phase separation, the acetonitrile phase was dehydrated with 1.0 g of sodium sulphate, filtered using a glass micro-fiber filter of 90 mm diameter and stored in 2 mL vials. The aqueous phase was filtered using polytetrafluoroethylene micro-fiber syringe filter of 13 mm diameter and 0.22-micron pore size and stored in 2 mL maxipense plastic vials prior to analysis. The acetonitrile phase was identified using gas chromatography (GC)-mass spectrometry, while the aqueous phase was quantified using a gas chromatography flame ionization detector (GC-FID).

Table 1. Information of paint samples collected in Lagos and Ibadan metropolises, Nigeria.

Serial No.	Manufacturer codes	Number of different paint colours collected	Number of paint samples collected	NIS-ISO registration
1	A	10	20	Yes
2	B	9	18	Yes
3	C	9	18	Yes
4	D	5	10	Yes
5	E	5	10	Yes
6	F	6	12	Yes
7	G	4	8	No
8	H	5	10	No
9	I	4	8	No
10	J	5	10	No
11	K	6	12	No
12	L	8	16	No
13	M	7	14	No
14	N	4	8	No

Note: Number of paint colours collected per manufacturer was based on availability and the most commonly patronized colour.

A recovery study was carried out using 2.5 mL of selected paint samples, which were measured into 50 mL polypropylene sterilized centrifuge tubes and extracted using the liquid-liquid extraction described above. Samples were spiked with different concentrations of film-forming agents (100-300 ppm) and analyzed using GC-FID. Recovery was calculated using Equation 1. The mean value of recovery of film-forming agents ranged from 92.5-96.4 % as presented in Table 2.

$$\% \text{ Recovery} = \frac{(A-B\times100\%\times D)}{z} \qquad \text{Eq. 1}$$

where,
A = spiked sample result.
B = unspiked sample result.
D = dilution factor.
Z = known concentration of analyte added .

Table 2. Recovery study of film-forming agents in paint samples.

VOCs	MS	MSD	MEAN±SD
EGBE	93.0	92.2	92.6±0.6
EGBEA	90.0	95.0	92.5±3.5
DEGBE	97.3	90.0	93.6±5.1
EGEEA	98.6	94.1	96.4±3.2

Abbreviations: : EGBE, ethylene glycol butyl ether; EGBEA, ethylene glycol butyl ether acetate; DEGBE, diethylene glycol butyl ether; EGEEA, ethylene glycol ethyl ether acetate;MS, matrix spike; MSD, matrix spike duplicate; SD, standard deviation.

Biodegradation Study

Cultivation of Micro-organisms

A suspended growth reactor (SGR) (2 L flask) comprised of sludge liquor, 8 bacteria and 6 fungi: *Bacillus polymyxa, Bacillus alvei, Bacillus cereus, Bacillus coagulans, Bacillus firmus, Bacillus laterosporus, Staphylococcus epidermidis and Staphylococcus kloosii; Aspergillus niger, Aspergillus fumigatus, Mucor racemosus, Rhizopus stolinifer, Cephalosporium* species, *and Penicillium* species, respectively, were incubated[39, 40].

Incubation was performed under aerobic conditions using artificial wastewater as a growth medium. The artificial wastewater was prepared using distilled water and minimal media or nutrients (consisting of 0.2 g/L yeast extract, 1.8 g/L ammonium chloride (NH_4Cl), 0.229 g/L dihydrogen potassium phosphate (KH_2PO_4), 4.5 g/L disodium hydrogen phosphate (Na_2HPO_4), 4.5 g/L calcium chloride dihydrate ($CaCl_2.2H_2O$) and 0.020 g/L magnesium (II) sulphate hexahydrate ($MgSO_4.7H_2O$)[41,42] in addition to 1 ml/L of trace elements solution (consisting of 0.5 g/100 mL manganese (II) sulphate monohydrate ($MnSO_4.H_2O$), 0.5 g/100 mL iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$), 0.078 6 g/100 mL copper (II) chloride dihydrate ($CuCl_2.2H_2O$), 0.245 g/100 mL cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$) and 0.439 g/100 mL zinc sulphate monohydrate ($ZnSO_4.H_2O$)). At the initial stage of feeding, this artificial wastewater was used for 7 days after which 0.5 g/L of sodium acetate (CH_3COONa) was added for further 7 days. This start-up period was needed to allow the biomass to be accustomed with the new feeding conditions[43]. Every day for 6 weeks, 1 L solution from the SGR was replaced with fresh artificial wastewater (50% volume exchange) in order to maintain the pH due to the metabolic products from biomass activity (a usual effect when the concentration of biomass is high).

Degradation of Ethylene Glycol Butyl Ether Acetate (EGBEA)

The degradation study was carried out in two stages; the first stage was to accustom the biomass to ethylene glycol butyl ether acetate for a month, followed by the second stage of biodegrading ethylene glycol butyl ether acetate at varying concentrations (5-20 ppm). Degradation experiments were carried out using five 250 mL flasks containing ethylene glycol butyl ether acetate as the only carbon source. The first flask was the control containing 3000 mg/L biomass and 50 mL minimal media. The second to the fifth flasks contained 3000 mg/L biomass, 50 mL minimal media and ethylene glycol butyl ether acetate in varying concentrations of 5, 10, 15 and 20 ppm, respectively. The flasks were stirred continuously at 400 rpm and sparged with air to provide dissolved oxygen. The system was maintained at 27±5 ºC and pH 7.0±0.5 Optical density of the contents in the flasks was measured using UV-spectrophotometer to check the uptake of ethylene glycol butyl ether acetate by the biomass. 20 mL of the solution in each flask were filtered using a PTFE micro-fibre syringe filter of 13-mm diameter and 0.22-micron pore size and analyzed for ethylene glycol butyl ether acetate present in the solution using GC-FID.

Instrument Operating Conditions

A Perkin Elmer-Clarus 680 GC model with a Perkin Elmer Packard MS was used for the identification of organic components present in the paint samples. Identification was based on molecular structure, molecular mass and calculated fragments. The column used was Agilent JW DB 5 non-polar column (30 m × 0.25 mm × 0.25 µm). Operating conditions: carrier gas was helium, flow rate was 1 mL/min at an atmospheric pressure of 12.9 psi and an initial flow of 26.3 cm/second; injection temperature was 200 °C; detector temperature was 280 °C; oven temperature program was 60 °C for 1.50 min and ramped to 280 °C at 10°C/min, where it remains there constant for 10.00 min. Interpretation of mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) with more than 62,000 patterns. The name, molecular weight and structure of each of the components of the paint samples were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. Electron ionization-mass spectroscopy (EI-MS) in selected ion monitoring mode (SIM) was used for the identification of the VOCs (Table 3). The spectrum of the unknown component was compared with the spectrum of the component stored in the Turbo mass software, and NIST MS search 2.0, 2008 was used and scanned (10:450) EI+ for identification as shown in Figure 1. The detected components with their retention time (RT), molecular formula and molecular weight (MW) are presented in Table 4.

Table 3. Monitoring ions (SIM) measurement used for the identification of film-forming agents in the paint samples.

Anti-freezing and film-forming agents	Monitor ions (m/z)
EGBE	57, 45, 41
EGBEA	43, 57, 41
EGEEA	43, 59, 72
DEGBE	45, 57, 29

The underlined number is the m/z of the ion used.

A Perkin Elmer – Clarus 500 GC model with Packard FID was used for the quantitative analysis of VOCs as well as to evaluate the degradation process. An Agilent JW DB-624UI column (30 m ×320 µm × 1.80 µm) was used. Ultrapure H_2 carrier gas was used with a flow rate of 45 mL/min. Operating conditions: injection temperature was 250 ºC, FID temperature was 280 ºC, column temperature was 50 ºC for 3 min ramped to 100 ºC at 6 ºC/min and then to 250 ºC at 10 ºC/min, where it was kept constant for 3 min. For the degradation study, all flasks were placed in an orbital shaker at 120 rpm at 37 ºC and subsequently analyzed using UV-VIS spectro-photometry (UV-1800 spectrophotometer). Microbial growth rate was measured at 600 nm, while EGBEA uptake was measured using GC-FID.

Table 4. Compounds detected in the paint samples.

Serial No.	Retention time (mins)	NIST	Molecular weight	Molecular formula	Class of compounds
1	2.28	2-Butanone, 3,3-dimethyl	100	$C_6H_{12}O$	Ketone
2	2.45	Propylene glycol	76.09	$C_3H_8O_2$	Glycol
3	3.20	Ethylene glycol butyl ether	118.17	$C_6H_{14}O_2$	Glycol ether
4	3.50	Ethylene glycol ethyl ether acetate	132.16	$C_6H_{12}O_3$	Ester
5	5.36	Diethylene glycol	106.12	$C_4H_{10}O_3$	Glycol
6	6.05	Ethylene glycol	62.07	$C_2H_6O_2$	Glycol
7	6.61	Ethylene glycol butyl ether acetate	160.21	$C_8H_{16}O_3$	Ester
8	7.30	Diethylene glycol butyl ether	162.23	$C_8H_{18}O_3$	Glycol ether
9	8.71	Triethylene glycol	150.17	$C_6H_{14}O_4$	Glycol
10	9.59	2,2,4-trimethyl-1,3-pentane diol diisobut	286	$C_{16}H_{30}O_4$	Texanol
11	9.85	Propanoic acid, 2-methyl-3-hydroxy-2,4	216	$C_{12}H_{24}O_3$	Texanol
12	11.66	Phenol 2,4-Bis (1,1-dimethyl ethyl)-	206	$C_{14}H_{22}O$	Phenol
13	12.20	1, Hexadecene	224	$C_{16}H_{32}$	Alkene
14	12.45	Furan, tetrahydro-2-methyl	86	$C_5H_{10}O$	Furan
15	13.30	Dihexyl fumarate	284	$C_{16}H_{28}O_4$	Ester
16	14.38	3 Eicosene	280	$C_{20}H_{40}$	Alkene
17	16.32	Dibutyl phthalate	278	$C_6H_{22}O_4$	Ester
18	18.05	1, Nonadecene	266	$C_{19}H_{38}$	Alkene
19	19.65	11- Tricosene	322	$C_{23}H_{46}$	Alkene
20	21.13	Oxalic acid, allyl tetradecyl ester	326	$C_{19}H_{34}O_4$	Ester

Multivariate Statistical Analysis

In order to develop a better understanding of the obtained data set, multivariate statistical analyses were performed. A multivariate statistic approach (principal component analysis, clustering analysis and correlation analysis) was adopted to assist the interpretation of the generated data[44,45].

Principal Component Analysis (PCA) is the general name for a technique which uses sophisticated underlying mathematical principles to transform a number of possibly correlated variables into a smaller number of variables called principal components[46,47]. Principal component analysis (PCA) can be applied in the study to get high-quality experimental results. Cluster analysis is a technique used for dividing similar objects into groups[48]. Analytical data can be calculated using the hierarchical clustering with SPSS software[49-51]. Correlation coefficient computes the pairwise associations for a set of variables and displays the results as a matrix. It is useful for determining the value of association of investigated variables[52].

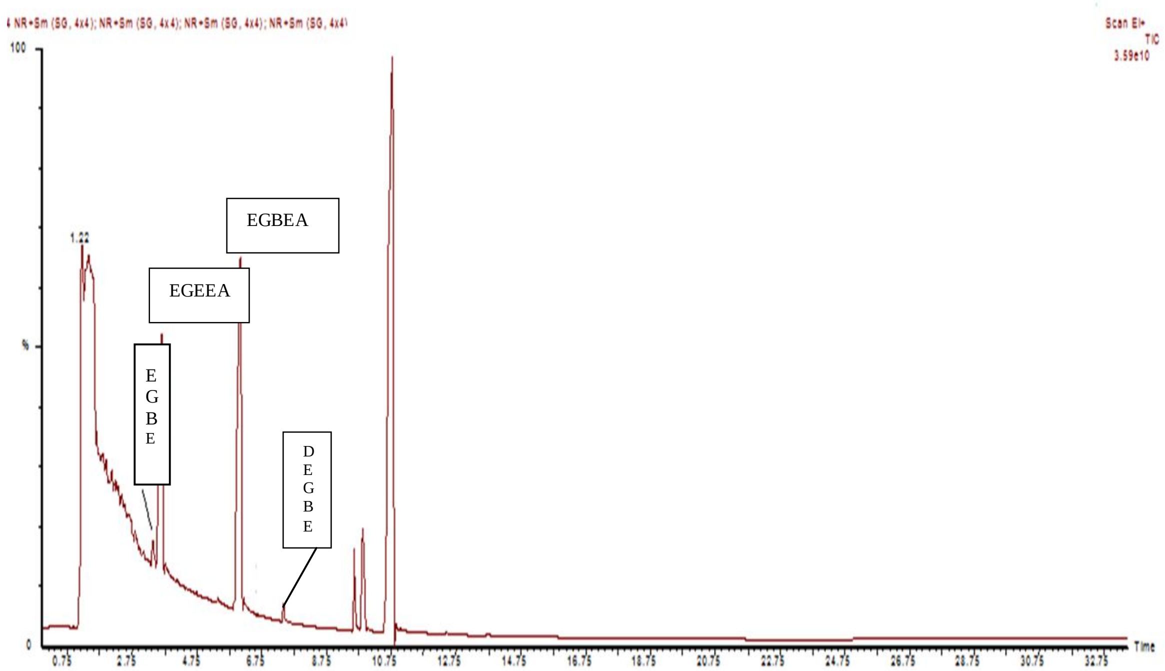

Figure 1. Chromatogram of FFAs by GC-MS.

Results and discussion

Identification of film-forming Agents in the Paint Samples

The GC-MS analysis of the paint samples revealed the presence of 20 organic compounds. The identification of the FFAs and some other VOCs present in the samples was confirmed using the retention time and molecular formula. Identified compounds include glycol ethers (37%) consisting of diethylene glycol dibutyl ether, diethylene glycol butyl ether, ethylene glycol ethyl ether; esters (15%) consisting of ethylene glycol ethyl ether acetate, ethylene glycol butyl ether acetate, oxalic acid- allyl tetradecyl ester, dibutyl phthalate, dihexyl fumarate; glycols (19%); alkenes (15%); phenols (3%); texanol (2%), ketone (1%) and furans (8%) (Figure 2).

Concentrations of FFAs in Paint Samples with Respect to Manufacturers

Fourteen selected manufacturers of paints were selected for the study: six registered with NIS- Nigerian Industrial Standard and ISO – International Organization for Standardization and eight unregistered manufacturers that produce different colours of paints. The concentrations of the 4FFAs….. considered in this work (ethylene glycol butyl ether (EGBE), diethylene glycol butyl ether (DEGBE), ethylene glycol butyl ether acetate (EGBEA) and ethylene glycol ethyl ether acetate (EGEEA)) in all studied paint samples are presented in Table 5, while Table 6 presents the mean concentrations ± SD. The variation in concentrations of the FFAs with respect to manufacturers is shown in Figure 3.

Diethylene Glycol Butyl Ether (DEGBE)

Diethylene glycol butyl ether (DEGBE) is a VOC with a boiling point of 230 ^{o}C. It irritates the eyes and may reach toxic levels after evaporation[53]. The concentrations of DEGBE in all paint samples ranged from 1,010-3,350 ppm (Table 5). The highest concentration was 3,350 ppm, obtained in paints produced by manufacturer F (a registered manufacturer). This was followed by 3,040 ppm and 2,760 ppm in paints produced by the same manufacturer. The lowest concentration was 1,010 ppm, obtained in a paint produced by manufacturer C (a registered manufacturer). The highest mean concentration of DEGBE was 2,590±210 ppm in paints produced by manufacturer F. This was followed by 2,100±87 ppm and 1,600±67 ppm in paints produced by manufacturer K (an unregistered manufacturer) and C, respectively, while the lowest mean concentration, 1,520±36 ppm, was obtained in paints produced by manufacturer B (a registered manufacturer) (Table 6).

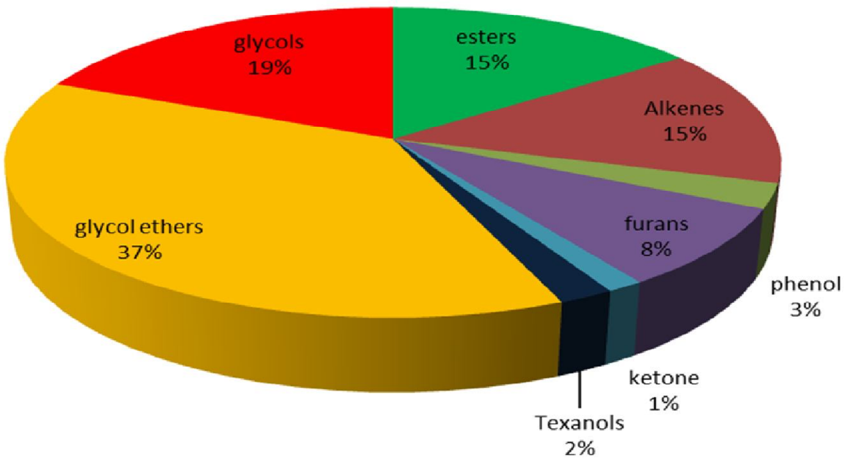

Figure 2. Percentage composition of identified compounds in the paint samples.

Table 5. Concentrations (ppm) of film-forming agents in paint samples with respect to manufacturers

		EGBE		EGBEA		DEGBE		EGEEA	
Manufacturer code	Primary colours in the paint samples	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
A	blue	1450	1550	1660	1730	NP	NP	NP	NP
	brown	1090	1170	1380	1120	NP	NP	NP	NP
	chocolate	1110	1030	2620	2870	NP	NP	NP	NP
	cream	1020	1060	2950	2920	NP	NP	NP	NP
	green	1390	1370	1550	1660	NP	NP	NP	NP
	grey	1260	1290	1990	2130	NP	NP	NP	NP
	pink	1180	1160	1580	1980	NP	NP	NP	NP
	red	1070	1100	1770	1940	NP	NP	NP	NP
	white	1030	1150	1760	1810	NP	NP	NP	NP
	yellow	1180	1170	2110	2270	NP	NP	NP	NP
B	blue	1620	1580	1490	1610	1480	1300	NP	NP
	green	1110	1110	2850	2820	1350	1380	NP	NP
	orange	1180	1180	2720	2640	1540	1490	NP	NP
	pink	1000	1140	3410	3310	1570	1650	NP	NP
	red	1120	1010	3220	3450	1610	1620	NP	NP
	white	1270	1320	3440	3360	1650	1770	NP	NP
	yellow	1290	1110	3320	3210	1730	1670	NP	NP
	brown	1360	1560	3800	3470	1250	1180	NP	NP
	cream	1170	1070	3050	2960	1580	1510	NP	NP

		EGBE		EGBEA		DEGBE		EGEEA	
Manufacturer code	Primary colours in the paint samples	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
C	blue	1210	1330	2390	2700	1180	1010	NP	NP
	brown	1440	1410	2680	2790	1750	1950	NP	NP
	chocolate	1120	1540	2950	3060	1850	1700	NP	NP
	green	1110	1490	2990	3300	1020	1260	NP	NP
	grey	1600	1350	2810	2710	1850	1950	NP	NP
	red	1360	1110	2510	2820	1500	1550	NP	NP
	violet	1050	1130	3080	3320	1520	1580	NP	NP
	white	1420	1110	2780	2480	1910	1600	NP	NP
	yellow	1170	1080	2210	2600	1700	1990	NP	NP
D	blue	1190	1090	2910	2900	NP	NP	NP	NP
	Cream	1140	1400	2800	2580	NP	NP	NP	NP
	Green	1340	1450	2200	2170	NP	NP	NP	NP
	Red	1290	1170	2670	2870	NP	NP	NP	NP
	White	1300	1300	2570	2490	NP	NP	NP	NP
E	Blue	1010	1200	NP	NP	NP	NP	NP	NP
	cream	1050	1300	NP	NP	NP	NP	NP	NP
	green	1270	1320	NP	NP	NP	NP	NP	NP
	white	1180	1090	NP	NP	NP	NP	NP	NP
	yellow	1520	1300	NP	NP	NP	NP	NP	NP

		EGBE		EGBEA		DEGBE		EGEEA	
Manufacturer code	Primary colours in the paint samples	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
F	blue	1270	1100	2430	2400	2580	2570	NP	NP
	Cream	1250	1310	2760	2540	2720	3040	NP	NP
	Green	1250	1170	2120	1940	2680	2600	NP	NP
	Red	1250	1050	2010	2260	2720	2760	NP	NP
	White	1340	1270	2900	2430	3350	2580	NP	NP
	Yellow	1330	1210	2320	2690	1980	1520	NP	NP
G	Blue	NP	NP	3300	3060	NP	NP	1220	1500
	Cream	NP	NP	3380	3520	NP	NP	1210	1340
	Green	NP	NP	3870	3610	NP	NP	1300	1530
	White	NP	NP	3030	3120	NP	NP	1020	1200
H	Blue	1300	1180	1900	1800	NP	NP	NP	NP
	Cream	1200	1190	2670	2590	NP	NP	NP	NP
	green	1380	1300	2110	1990	NP	NP	NP	NP
	pink	1110	1180	3320	3600	NP	NP	NP	NP
	white	1150	1060	1900	2300	NP	NP	NP	NP
I	blue	1200	1400	2890	2800	1490	1480	NP	NP
	cream	1560	1510	3620	3110	1570	1870	NP	NP
	green	1230	1100	2780	2570	1500	1400	NP	NP
	white	1560	1390	3670	3770	1860	1550	NP	NP

		EGBE		EGBEA		DEGBE		EGEEA	
Manufacturer code	Primary colours in the paint samples	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
J	blue	1080	1100	2000	2060	NP	NP	NP	NP
	Cream	1150	1090	1580	1350	NP	NP	NP	NP
	Green	1190	1070	1910	1830	NP	NP	NP	NP
	Red	1130	1100	1670	1580	NP	NP	NP	NP
	White	1120	1080	1840	1770	NP	NP	NP	NP
K	Blue	1090	1190	2030	2110	1820	1800	NP	NP
	chocolate	1560	1200	2790	2340	2200	1860	NP	NP
	Cream	1120	1030	1890	1870	1860	1740	NP	NP
	Green	1030	1160	1490	1670	1920	2130	NP	NP
	Red	1370	1080	3070	3180	2740	2690	NP	NP
	White	1220	1210	1980	1770	2350	2130	NP	NP
L	Blue	1300	1150	2040	2380	NP	NP	NP	NP
	Brown	1150	1060	2710	2540	NP	NP	NP	NP
	chocolate	1160	1140	2330	2900	NP	NP	NP	NP
	cream	1160	1040	2060	1900	NP	NP	NP	NP
	green	1100	1230	1830	1660	NP	NP	NP	NP
	orange	1080	1140	1990	1920	NP	NP	NP	NP
	pink	1130	1040	2580	2900	NP	NP	NP	NP
	white	1030	1120	2400	2130	NP	NP	NP	NP

		EGBE		**EGBEA**		**DEGBE**		**EGEEA**	
Manufacturer code	Primary colours in the paint samples	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
M	blue	1040	1080	2530	2350	NP	NP	NP	NP
	Cream	1170	1240	2480	2610	NP	NP	NP	NP
	Green	1040	1140	2740	2550	NP	NP	NP	NP
	Orange	1010	1050	2740	3060	NP	NP	NP	NP
	Pink	1390	1070	3000	2810	NP	NP	NP	NP
	Red	1250	1160	2800	2800	NP	NP	NP	NP
	White	1170	1060	2870	2400	NP	NP	NP	NP
N	Blue	1860	1460	2510	2840	NP	NP	1970	1990
	cream	1800	1850	2710	2800	NP	NP	2790	2900
	green	1360	1360	3010	2890	NP	NP	2150	2000
	white	1380	1550	2940	2900	NP	NP	2000	2090

Note: NP= not present.

Table 6. Mean concentrations (±SD) in ppm of film-forming agents in paint samples with respect to manufacturers.

		Concentrations of film-forming agents			
S/N	Manufacturer code	EGBE	EGBEA	DEGBE	EGEEA
1	A	1,190±27	1,990±80	NP	NP
2	B	1,230±52	3,010±66	1,520±36	NP
3	C	1,270±64	2,620±69	NP	NP
4	D	1,280±99	2,790±78	1,600±67	NP
5	E	1,220±61	NP	NP	NP
6	F	1,240±41	2,400±110	2,590±210	NP
7	G	NP	3,360±57	NP	1,290±45
8	H	1,200±29	2,420±100	NP	NP
9	I	1,370±46	3,160±130	1,590±140	NP
10	J	1,110±27	1,760±51	NP	NP
11	K	1,190±95	2,180±110	2,100±87	NP
12	L	1,130±27	2,270±110	NP	NP
13	M	1,130±67	2,690±100	NP	NP
14	N	1,580±128	2,830±88	NP	2,240±36
Range		1,110-1,580	1,760-3,350	1,520-2,590	1,290-2,240

NP means not present; EU permissible limit of VOCs is 500 ppm.

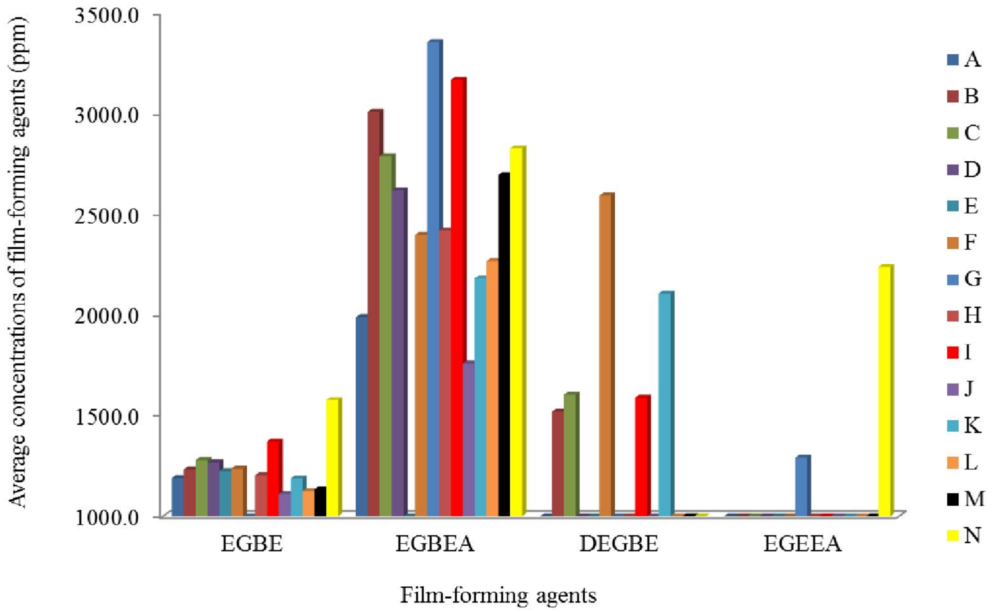

Figure 3. Variation in the concentrations of film-forming agents in the paint samples with respect to manufacturers.

Ethylene Glycol Butyl Ether (EGBE)

Ethylene glycol butyl ether (EGBE) is a VOC with a boiling point of 156 °C. It irritates the eyes and skin and affects the central nervous system, kidney and liver[54]. The concentrations of EGBE in all the paint samples ranged from 1,000-1,860 ppm (Table 5). The highest concentration of EGBE was 1,860 ppm, obtained in a paint produced by manufacturer N (an unregistered manufacturer). This was followed by 1,850 ppm and 1,800 ppm in products produced by the same manufacturer. The lowest concentration was 1,000 ppm, obtained in a paint produced by manufacturer N (an unregistered manufacturer). The highest mean concentration of EGBE was 1,580±128 ppm in paints produced by manufacturer N. This was followed by 1,370±46 ppm in paints produced by manufacturer I (an unregistered manufacturer) and 1,280±99 ppm in paints produced by manufacturer C (a registered manufacturer), while the lowest mean concentration was 1,110±27 ppm in paints produced by manufacturer J (unregistered manufacturer) (Table 6). The concentrations of EGBE in all samples were above the EU permissible limit of 500 ppm except in paint samples produced by manufacturer G (unregistered manufacturer). It was reported that there is a possibility that evaporated EGBE reaches a toxic level. Caution for use should be indicated in the product label[15].

Ethylene Glycol Butyl Ether Acetate (EGBEA)

Ethylene glycol butyl ether acetate (EGBEA), also known as 2-butoxylethanol acetate, irritates the eye, skin, nose and throat and causes hemolysis, hematuria (blood in the urine), central nervous system depression, headache and vomiting[54,55]. The concentrations of ethylene glycol butyl ether acetate (EGBEA) ranged in all samples from 1,120 to 3,870 ppm (Table 5). The highest concentration of EGBEA was 3,870 ppm found in paints produced by manufacturer G (an unregistered manufacturer). This was followed by 3,800 ppm and 3,770 ppm in products produced by manufacturer B (a registered manufacturer) and manufacturer I (an unregistered manufacturer), respectively. The lowest concentration was 1,120 ppm, obtained in a paint produced by manufacturer A (a registered manufacturer). The highest mean concentration of EGBEA with respect to manufacturers was 3,360±57 ppm in paints produced by manufacturer G. This was followed by 3,160±130 ppm and 3,010±66 ppm in paints produced by manufacturers I and B, respectively, while the lowest mean concentration was 1,760±51 ppm in paints produced by manufacturer J (an unregistered manufacturer) (Table 6). The concentrations of EGBEA were in all samples above the permissible 500-ppm limit of EU except in paint samples produced by manufacturer E (a registered manufacturer). EGBEA has a boiling point of 188 °C.

Ethylene Glycol Ethyl Ether Acetate (EGEEA)

Ethylene glycol ethyl ether acetate (EGEEA), also known as 2-Ethoxylethyl acetate, irritates the eyes and nose and causes vomiting, kidney damage and paralysis in humans. In animals, it causes reproductive and teratogenicity effects[56].

The concentrations of ethylene glycol ethyl ether acetate (EGEEA) ranged in all samples from 1,020 to 2,900 ppm (Table 5). The highest concentration of EGEEA was 2,900 ppm, obtained in paints produced by manufacturer N (an unregistered manufacturer). The lowest concentration was 1,020 ppm, obtained in a paint produced by manufacturer G (an unregistered manufacturer). EGEEA was not present in the paint samples produced by most of the manufacturers except by manufacturers G and N.

The highest mean concentration of EGEEA was 2,240±36 ppm, in paints produced by manufacturer N. while the lowest mean concentration was 1,290±45 ppm in paints produced by manufacturer G (a registered manufacturer), Table 6. EGEEA is a prohibited VOC in indoor and outdoor paints by the EU. It has a boiling point of 156 °C.

Concentrations of Film-forming Agents in Paint Samples with Respect to Colours

The concentrations of FFAs with respect to paint colours are shown in Table 7; the variation of the FFA concentrations with respect to colours is presented in Figure 4.

Table 7. Mean concentrations (±SD) in ppm of film-forming agents in paint samples with respect to colours.

	Concentrations of film-forming agents			
Paint colours	EGBE	EGBEA	DEGBE	EGEEA
blue	1,270±69	2,340±82	1,670±62	1,670±130
brown	1,280±52	2,560±69	1,530±60	NP
chocolate	1,230±140	2,730±140	1,900±95	NP
cream	1,250±57	2,610±92	1,980±87	2,060±14
green	1,230±69	2,390±57	1,720±64	1,750±41
grey	1,370±110	2,410±20	1,900±68	NP
orange	1,110±21	2,510±100	1,520±31	NP
pink	1,140±81	2,850±82	1,610±59	NP
red	1,160±70	2,540±68	2,150±14	NP
violet	1,090±60	3,200±175	1,550±42	NP
white	1,220±57	2,570±110	2,080±178	1,580±46
yellow	1,240±59	2,590±100	1,760±190	NP
Range	1,090-1,370	2,340-3,200	1,520-2,150	1,580-2,060

Note: NP = not present.

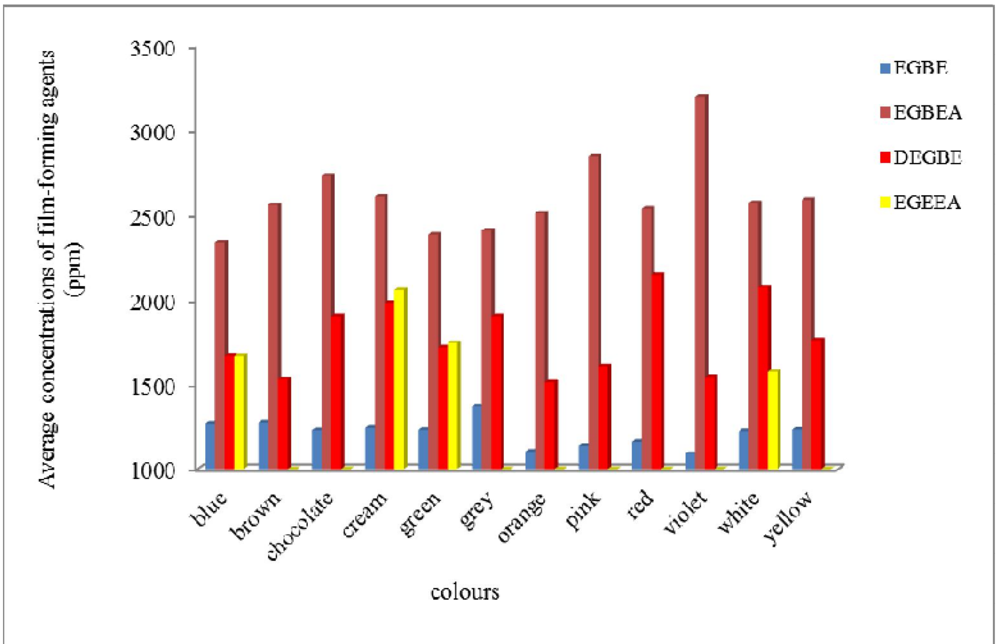

Figure 4. Variation in concentrations of film-forming agents in the paint samples with respect to paint colours.

Diethylene Glycol Butyl Ether (DEGBE)

The concentrations of diethylene glycol butyl ether in all the paint samples with respect to colours ranged from 1,010 ppm (blue) to 3,350 ppm (white). The highest concentration was 3,350 ppm in a white coloured paint, followed by 3,040 ppm and 2,760 ppm in a cream and a red coloured paint, respectively. The highest mean concentration with respect to colours was 2,150±14 ppm in a red coloured paint, followed by 2,080±178 ppm in a white and 1980±87 ppm in a cream coloured paint. The lowest mean concentration was 1,520±31 ppm, obtained in an orange coloured paint.

Ethylene Glycol Butyl Ether (EGBE)

The concentrations of ethylene glycol butyl ether with respect to colours ranged in all samples from 1,000 ppm (pink) to 1,860 ppm (blue). The highest concentration was 1,860 ppm in a blue coloured paint, followed by 1,850 ppm and 1,800 ppm in cream coloured paints. The highest mean concentration with respect to colours was 1,370±110 ppm in a grey coloured paint, followed by 1,280±52 ppm in a brown and 1,270±69 ppm in a blue coloured paint. The lowest mean concentration was 1,090±60 ppm, observed in a violet coloured paint.

Ethylene Glycol Butyl Ether Acetate (EGBEA*)*

The concentrations of ethylene glycol butyl ether acetate in all the paint samples with respect to colours ranged from 1,120 (brown) to 3,870 ppm (green). The highest concentration was 3,870 ppm in a green coloured paint, followed by 3,800 ppm in a brown and 3,770 ppm in a white coloured paint. The highest mean concentration with respect to colours was 3,200±175 ppm in a violet coloured paint, followed by 2,850±82 ppm in a pink and 2,730±140 ppm in a chocolate coloured paint. The lowest mean concentration was 2,340±82 ppm, obtained in a blue coloured paint.

Ethylene Glycol Ethyl Ether Acetate (EGEEA)

The concentrations of ethylene glycol ethyl ether acetate in all samples with respect to colours ranged from 1,020 (white) to 2,900 ppm (creamy). The highest concentration was 2,900 ppm in a cream coloured paint, followed by 2,790 ppm in another cream paint produced by another manufacturer and 2,150 ppm in a green coloured paint. The highest mean concentration with respect to colours was 2,060±14 ppm in a cream coloured paint, followed by 1,750±41 ppm in a green and 1,670±130 ppm in a blue coloured paint. The lowest mean concentration was 1,580±46 ppm, obtained in a white coloured paint.

The order of FFAs present with respect to paint colours is:

EGBE: grey > brown > blue > cream > yellow > green > white > red > pink > orange > violet.

EGBEA: violet > pink > chocolate > cream > white > brown > red > orange > grey > green > blue.

DEGBE: red > white > cream > chocolate > grey > yellow > green > blue > pink > violet > brown.

EGEEA: cream > green > blue > white.

Results of Degradation Study

The Monod kinetics model is a mathematical relationship for micro-organism growth and substrate utilization. In the Monod equation, $\mu = \mu_{max}. [S/(S+K_s)]$, μ is the specific growth rate of the micro-organisms, μ_{max} is the maximum specific growth rate of the micro-organisms, S is the concentration of the limiting substrate for growth and K_s is the half-velocity constant which can be calculated from the slope and intercept in the plot of 1/μ versus 1/S (K_s=slope/intercept). The results of the degradation of EGBEA (most commonly used FFA in paints) are presented in Table 8 and Figure 5 (slope=4.008, R^2=0.9796; intercept =1.6595). The microbial growth rate was rapid within 4 hours for all the concentrations of ethylene glycol butyl ether acetate (EGBEA) and the uptake efficiency was very gradual at 20 ppm as shown in Figure 6. The accelerated (log) phase was marked with significant increase in cell population doubling at regular interval of time up to 48 hours for the depletion of 5-15 ppm of EGBEA. As the concentration of EGBEA increases, the growth rate of micro-organisms increases. At 20 ppm of EGBEA, the stationary phase was observed (cell growth rate equals cell death rate), which implied its possible bioaccumulation over time. The biodegradation study of EGBEA fitted into a typical Monod kinetics model for substrate utilization as shown in Figure 7.

Table 8. Degradation of EGBEA using mixed culture.

Concentrations of EGBEA in ppm (S)	Growth rate (μ =α)	1/μ	1/S	$\mu = \mu_{max.} [S/(S+K_s)]$
5	0.41	2.43902	0.2	0.40632
10	0.47	2.12766	0.1	0.48537
15	0.52	1.92308	0.06667	0.51902
20	0.55	1.81818	0.05	0.53766

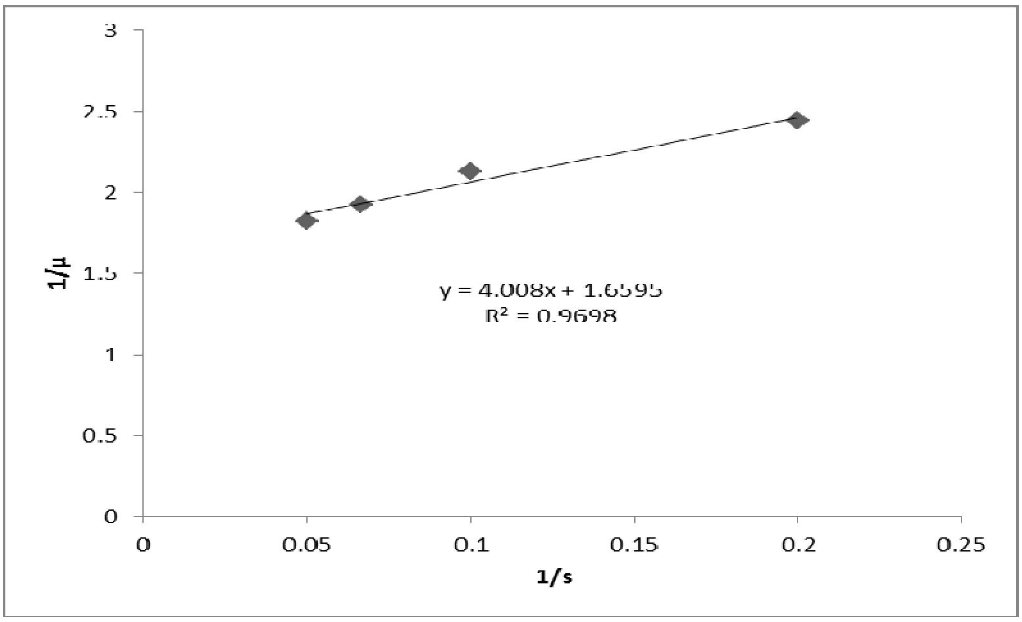

Figure 5. Linearized Monod equation for the biodegradation of EGBEA.

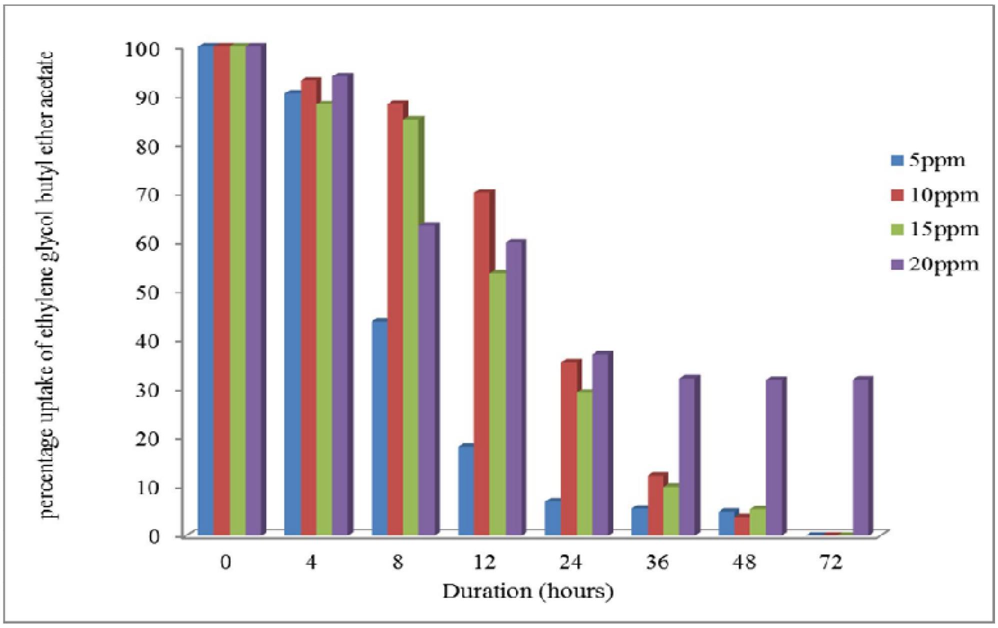

Figure 6. Percentage uptake in the degradation of EGBEA.

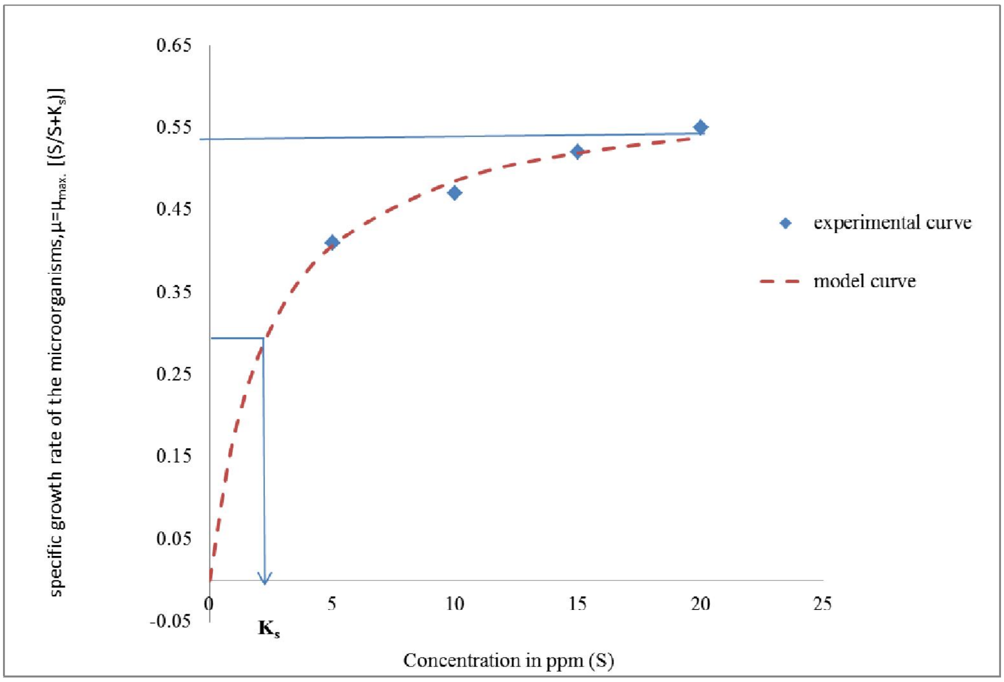

Figure 7. The Monod kinetics profile of EGBEA.

Results of Multivariate Statistical Analysis

The correlation coefficient was used to assess a possible linear association between two continuous variables. EGBEA had a positive correlation with DEGBE and EGEEA due to their film-forming properties and durability under diverse conditions and temperatures in paints (Table 9).

Table 9. Correlation coefficient of film-forming in the paint samples.

	Manufacturers	Colours	EGBE	EGBEA	DEGBE	EGEEA
Manufacturers	1	-0.088	-0.008	0.047	-0.201	0.321**
Colour		1	-0.037	0.049	0.094	-0.077
EGBE			1	-0.167	0.204	-0.159
EGBEA				1	0.262*	0.235*
DEGBE					1	-0.230*
EGEEA						1

Note: ** significant at 0.01; * significant at 0.05.

PCA successfully reduced the dimensionality of the data set and two principal components were extracted. This indicated that two main controlling factors influenced the quality of paints sampled, which were principally, colour and manufacturer. Corresponding components, variable loadings and the variances are presented in Table 10a. Only principal components (PCs) with eigenvalues greater than 1 were considered. PCA of the whole data set yielded 2 data sets explaining 67.04% of the total variance. First component was responsible for 35.47% of the variance and was best represented by EGBE while the second component, which is orthogonal to the first PC, explains 31.56% of the variance and was dominated by EGBEA. These film-forming agents are extensively used as solvents in surface coatings, such as lacquers, enamels, varnishes and latex paints, in paint thinners, in paint stripping formulations and inks and in degreasers as well as industrial and household cleaners. The rotated component matrices clearly indicate good relationship of the compounds and are consequently well represented by these two principal components as shown in Figure 8 and also boldly indicated in Table 10b. EGBEA and EGEEA are esters. They are more polar than ethers, but less polar than alcohols. Their ability to participate in hydrogen bonding confers some water-solubility. Consequently, esters are more volatile than carboxylic acids of similar molecular weight. DEGBE and EGBE are glycol ethers, a group of solvents based on alkyl ethers of ethylene glycol or propylene glycol commonly used in paints and cleaners. These solvents have typically higher boiling points, in addition to the favourable solvent properties of lower-molecular weight ethers and alcohols.

Cluster analysis (CA) was performed on the data using the between-groups linkage method and squared Euclidean distance applying hierarchical clustering with the SPSS software. Figure 9 shows the CA results for the film-forming agents in the paint samples as a dendrogram of two clusters. In the first one, EGBE, DEGBE and EGEEA are well correlated with one another. These compounds were found in similar colours (blue, creamy, green, white) and manufacturers. In the second cluster, only EGBEA is present. EGBEA was found in all the paint samples except those produced by manufacturer E. This explains its separation from the other compounds. Human exposure to these compounds can lead to nervous depression, nausea, vomiting and sometimes diarrhoea, prominent headache, abdominal and lumbar pain and cost vertebral angle tenderness, transient polyuria, anuria and acute renal failure. The CA results are not substantially different from those of PCA.

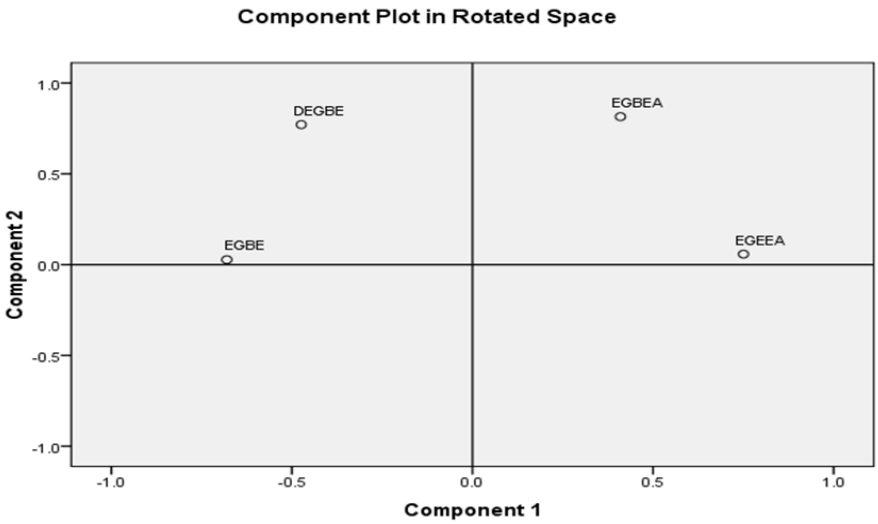

Figure 8. Rotated component matrices of film-forming agents.

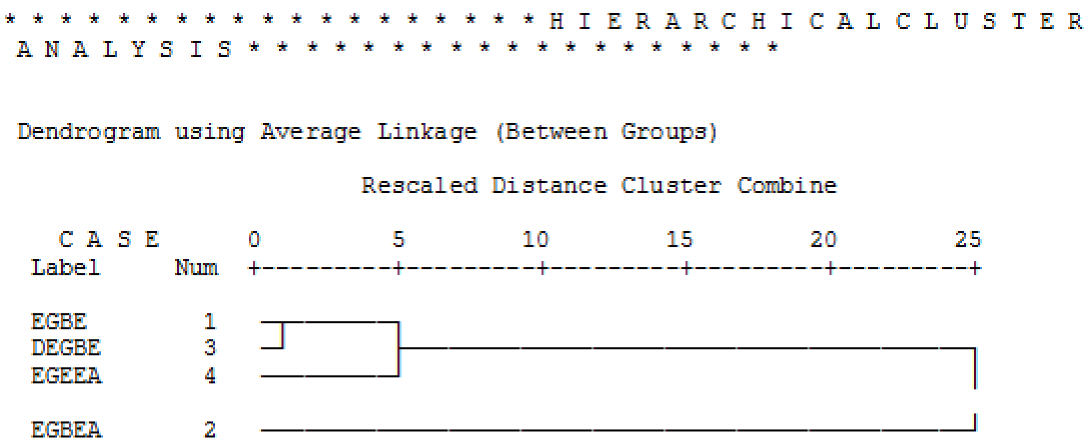

Figure 9. Dendrogram results of hierarchical cluster analysis for film-forming agents.

Table 10. Total variance and component matrices for film-forming agents.

(a) Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	1.419	35.474	35.474	1.419	35.474	35.474	1.419	35.467	35.467
2	1.262	31.562	67.036	1.262	31.562	67.036	1.263	31.569	67.036
3	0.843	21.066	88.102						
4	0.476	11.898	100						

(b) Component matrices

Compounds	Component matrix		Rotated component matrix	
	PC1	PC2	PC1	PC2
EGBE	0.681	-0.002	**-0.680**	0.027
EGBEA	-0.374	0.832	0.410	**0.815**
DEGBE	0.507	0.750	-0.474	**0.771**
EGEEA	-0.747	0.090	**0.751**	0.058

Extraction method: PCA; Rotation method: Varimax with Kaiser normalization; Bold figures indicate absolute values > 0.5 of parameters with strong loading values.

Conclusions

This study identifies and assesses four film-forming agents; namely, ethylene glycol butyl ether, ethylene glycol butyl ether acetate, diethylene glycol butyl ether and ethylene glycol butyl ether in paints marketed in Nigeria. The levels of all four FFAs in the paint samples investigated in this study were above the EU permissible limit of 500 ppm. The concentrations were higher in the products produced by unregistered manufacturers when compared to those produced by registered manufacturers. As reported by the European Union[57]. ethylene glycol butyl ether acetate is a prohibited film-forming agent in paints. In this study, the highest concentration was 3,870 ppm of EGBEA in a green coloured paint produced by manufacturer I (unregistered). Hence, water-based paints marketed in Nigeria contained high levels of FFAs, which are detrimental to human health and environment. There is therefore a need to put a regulation in place for the control of DEGBE, EGBE, EGEEA and the prohibition of EGBEA in paints to safeguard public health and the environment. VOC components and other substances should be clearly indicated on paint products.

The biodegradation of the commonly used and toxic EGBEA was found to fit into the typical Monod kinetics model for substrate utilization. The microbial growth rate increased with increased concentrations of EGBEA. Multivariate statistics analyses (CA coupled with PCA) were used to improve the interpretation of the data sets. The compounds were grouped based on similarities and variations in the data sets from the manufacturers and colours were examined.

Acknowledgements

The authors are grateful to The World Academy of Sciences for the Advancement of Science in Developing Countries and the Council of Scientific and Industrial Research for funding part of this research (FR number: 3240275040). The director of National Environmental Engineering Research Institute, Nehru Marg, Nagpur, India is highly appreciated for his support.

References

[1] Kirkeskov, L.; Witterseh, T.; Funch, L. W.; Kristiansen, E.; Mølhave, L.; Hansen, M. K.; Knudsen, B. B. *Indoor Air J*. **2009**, *19*:45-57.

[2] Nicolle, J.; Desauziers, V.; Mocho, P. *J. Chromatogr. A* **2008**, *1208*, 10-15.

[3] Han, K. H.; Zhang, J. S.; Wargocki, P.; Knudsen, H. N.; Guo, B. *Indoor Air* **2010**, *20*, 341-354.

[4] Jia, C.; Batterman, S.; Godwin, C.; Charles, S.; Chin, J. Y. *Indoor Air* **2010**, *20*, 357-369.

[5] Ho, D. X.; Kim, K. H.; Ryeul Sohn, J.; Hee Oh, Y.; Ahn, J. W. *The Scientific World J*. **2011**, *11*, 1597-1622.

[6] Kwon, K. D.; Jo, W. K.; Lim, H. J.; Jeong, W. S. *Environ. Sci. Pollut. Res. Int.* **2008**, *15*, 521-526.

[7] Fromme, H.; Dietrich, S.; Heitmann, D.; Dressel, H.; Diemer, J.; Schulz, T.; Jörres, R. A.; Berlin, K.; Völkel, W. *Food Chem. Toxicol*. **2009**, *47*, 1636-1641.

[8] Kabir, E.; Kim, K. H. *J. Hazard. Mater.* **2011**, *188*, 443-454.

[9] The Essential Chemistry Industry. Paint. 2013. http://www.essentialchemicalindustry.org/materials-and-applications/paints.html. Accessed 12 January, 2018.

[10] Odior, A. O. *Industrial Engineering Letters* **2012**, *2*, 18-26.

[11] Beetseh, C. I.; Oragbe, D. *Chem. Mat. Res*. **2013**, *3*, 53-60.

[12] Adamu, A. K.; Yakubu, M. K.; Sunmonu, O. K. Characterization of Emulsion Paints formulated using Reactive–Dyed Starch as a Pigment. International Conference on Biological, Chemical and Environmental Sciences. 2014. http://iicbe.org/upload/8184C614005.pdf

[13] Bessonneau, V.; Mosqueron, L.; Berrubé, A.; Mukensturm, G.; Buffet-Bataillon, S.; Gangneux, J. P.; Thomas, O. *PloS one* **2013**, *8*, p.e55535.

[14] European Union (2004) "Directive 2004/42/CE of the European Parliament and of the Council," EUR-Lex, European Union Publications Office. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32004L0042. Accessed 19. Dec., 2017.

[15] Nakashima, H.; Nakajima, D.; Takagi, Y.; Goto, S. *J Health Sc.* **2007**, *53*, 311-319.

[16] Jeng H. A.; Lee, I.; Gau, Y. Y.; Yang, C. T. *J Environ Health.* **2006**, *69*, 20.

[17] Wang, F.; Liu, F.; Liu, H.; Chen, W.; Si, X.; Ma, X. *Inhal Tox* **2016**, *28*, 164-169.

[18] Tung- Sheng, S.; Yu-Chieh, K.; Ro-Han, L.; Saou- Hsing, L.; Ho-Yuan, C.; Tzu-Chieh, C. *American J Industrial Med.* **2009**, *52*, 654-661.

[19] Ching-Hui, L.; Saou-Hsing, L.; Shyi-Shiaw, J.; Wei-Tung, C.; Tung-Sheng, S.; Hong, I. C. *Industrial Health J.* **2008**, *46*, 463-469.

[20] Yao, M.; Zhang, Q.; Hand, D. W.; Perram, D.; Taylor, R. *J Air Waste Manag Assoc.* **2009**, *59* (1), 31-36.

[21] Al-Khulaifi, N. M.; Al-Mudhaf, H. F.; Alenezi, R.; Abu-Shady, A. I.; Selim, M. I. *J Environ. Pro.* **2014**, *5*, 310-326.

[22] Chang, T. Y.; Lin, S. J.; Shie, R. H.; Tsai, S. W.; Hsu, H. T.; Tsai, C. T.; Kuo, H. W.; Chiang, C. F.; Lai, J. S. *J Air Waste Manag Assoc.* **2010**, *60* (1), 55-62,

[23] Hodgson, A. T.; Daisey, J. M.; Grot, R. A. *J. Air Waste Manag. Assoc.* **1991**, *41*(11), 1461-1468

[24] Betha, R.; Selvam, V.; Blake, D. R.; Balasubramanian, R. *J Air Waste Manag Assoc.* **2011**, *61* (11), 1093-1101

[25] Chen, K. S.; Lai, C. H.; Ho, Y. T. *J Air Waste Manag Assoc.* **2003**, *53* (1), 102-112,

[26] Censullo, A. C.; Jones, D. R.; Wills, M. T. *J. Coat. Technol.* **1997**, *69*, 33-41.

[27] Fortmann, R.; Roache, N.; Chang, J. C. S.; Guo, Z. *J Air Waste Manag Assoc.* **1998**, *48* (10), 931-940.

[28] Cox, S. S.; Little, J. C.; Hodgson, A. T. *J Air Waste Manag Assoc.* **2001**, *51* (8), 1195-1201.

[29] Vander Pol, S. S.; Becker, P. R.; Ellisor, M. B.; Moors, A. J.; Pugh, R. S.; Roseneau, D. G. *Environ. Poll.* **2009**, *157*, 755-762.

[30] Chauhan, A.; Goyal, M. K.; Chauhan, P. *J Analytical Bioanalytical Tech.* **2014**, 5 (6), 1000222.

[31] Mahanty, A.; Maji, S. R.; Ganguly, S.; Mohanty, B. P. *J Analytical Bioanaly. Tech.* **2015**, *6* (2), 1-4.

[32] Teklu, B. M.; Adriaanse, P. I.; Van den Brink, P. *J. Chemo.* **2016**, *161*, 280-291.

[33] Yeo, H.; Youn, K.; Kim, M.; Yun, E.Y.; Hwang, J.S.; Jeong, W. S.; Jun, M. *Preventive Nutri. Food Sci.* **2013**, *18*,150.

[34] Abdel-Aal E, I.; Haroon, A. M.; Mofeed J. *The Egyp J Aquatic Res.* **2015**, *41*, 233-246.

[35] Pragst, F.; Balikova, M. A. *Clinica Chimica Acta.* **2006**, *370*, 17-49.

[36] Scott-Ham, M.; Burton, F. C. *J Clinical Forensic Med.* **2005**, *12*, 175-186.

[37] Sethi, S.; Nanda, R.; Chakraborty, T. *Clin Microbiol Rev.* **2013**, *26*, 462-475.

[38] Nair, H.; Woo, F.; Hoofnagle, A. N.; Baird, G. S. *J Tox.* **2013**:Article ID 329407, 7.

[39] Rolfe, M. D.; Rice, C. J.; Lucchini, S.; Pin, C.; Thompson, A.; Cameron, A. D.; Alston, M.; Stringer, M. F.; Betts, R. P.; Baranyi, J.; Peck, M. W. *J Bact.* **2012**, *194*, 686-701.

[40] Shinkafi, S. A.; Haruna, I. *Inter J Current Microb. Appl. Sci.* **2013**, *2*, 314-324.

[41] Yang, S.F.; Lin, C. F.; Wu, C. J.; Ng, K. K.; Lin, A. Y. C.; Hong, P. K. A. *Water Res.* **2012**, *46*, 1301-1308.

[42] Fontaina, E. F.; Pinho, I.; Carballa, M.; Omil, F.; Lema, J. M. *Biodegrad.* **2013**. *24*, 165-177.

[43] Suarez, S.; Lema, J. M.; Omil, F. *Water Res.* **2010**, *44*, 3214-3224.

[44] Oliva, S. R.; Espinosa, A. J. F. *Microchem. J.* **2007**, *86*, 131–139.

[45] Tuncer, G. T.; Tuncel, S. G.; Tuncel, G.; Balkas, T. I. *Water Sci. and Tech.* **1993**, *28*, 59–64.

[46] Abdi, H.; Williams, L. J. *Computational Statistics* **2010**, *2* (4), 433-459.

[47] Richardson, M. **2009**. Principal component analysis. URL: http://people. maths. ox. ac. uk/richardsonm/SignalProcPCA. pdf (Accessed 19. Dec., 2017).

[48] Jain, A.K. *Pattern recog. Lett.* **2010**, *31* (8), 651-666.

[49] Hsu, J. J.; Finkelstein, D. M.; Schoenfeld, D. A. *PloS one*, **2015**, *10* (11), e0141874.

[50] Pok, G.; Liu, J. C. S.; Ryu, K. H. *Bioinformation* **2010**, *4* (8), 385.

[51] Tang, D.; Wang, M.; Zhou, W. *Int. J. Hybrid Intell. Systems*, **2016,** *13*(1), 27-37.

[52] Mukaka, M. M. *Malawi Med. J.* **2012,** *24* (3), 69-71.
[53] National Institute for Occupational Safety and Health (NIOSH). Diethylene glycol butyl ether. *International Chemical Safety Cards* (ICSC), **1994**. https://www.cdc.gov/niosh/ipcneng/neng0788.html. Accessed 19. Dec., 2017.
[54] National Institute for Occupational Safety and Health (NIOSH). Criteria for a Recommended Standard: Occupational Exposure to Ethylene Glycol Monobutyl Ether and Ethylene Glycol Monobutyl Ether Acetate **1990**. Accessed 19. Dec., 2017.
[55] Agency for Toxic Substances and Disease Registry (ATSDR). 2-Butoxyethanoland 2-Butoxylethanol Acetate, **1998**. https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=347&tid=61. Accessed 19. Dec., 2017.
[56] National Institute for Occupational Safety and Health (NIOSH). 2-Ethoxyethyl Acetate. International Chemical Safety Cards (ICSC). 2008.
[57] Revision of European Ecolabel and Development of Green Public Procurement Criteria for Indoor and Outdoor Paints and varnishes. Consultation Document to Analyse the Scope and Existing Criteria for Paints and Varnishes. 2011.
[58] http://susproc.jrc.ec.europa.eu/paints/docs/Consultation%20document%20for%20Paints%20Ecolabel%20GPP%20criteria.pdf. Accessed 19. Dec., 2017.